

Application Serial No.: 09/761,782  
Supplemental Response to Office Action of May 29, 2003

### AMENDMENTS TO THE CLAIMS

1. (Previously Presented) An isolated DNA molecule encoding a small subunit of acetohydroxy acid synthase isozyme III originating from *Escherichia coli*, which has a mutation selected from the group consisting of:

a) a mutation that replaces the serine at amino acid number 17 in SEQ ID NO: 2 with an amino acid other than serine and

b) a mutation that replaces both (i) the serine residue at amino acid number 17 in SEQ ID NO: 2 with an amino acid other than serine and (ii) the glycine residue at the amino acid number 14 in SEQ ID NO: 2 with an amino acid other than glycine,

wherein the unmutated sequence of acetohydroxy acid synthase isozyme III is SEQ ID NO:2.

2. (Previously Presented) The isolated DNA according to claim 1, wherein the mutation at amino acid number 17 replaces serine with a phenylalanine residue and the mutation at the amino acid number 14 replaces glycine with an aspartic acid residue.

3. (Canceled)

4. (Currently Amended) An isolated DNA encoding a large subunit and a mutated small subunit of acetohydroxy acid synthase isozyme III originating from *Escherichia coli*,

wherein the unmutated sequence of the small subunit of acetohydroxy acid synthase isozyme III is SEQ ID NO:2 and wherein said small subunit has a mutation that replaces the glycine residue at amino acid number 14 in SEQ ID NO: 2 with an amino acid other than glycine and has at least one mutation selected from the group consisting of:

a) a mutation that replaces the serine residue at amino acid number 17 in SEQ ID NO: 2 with an amino acid other than serine,

Application Serial No.: 09/761,782  
Supplemental Response to Office Action of May 29, 2003

b) a mutation that replaces the asparagine residue at amino acid number 29 in SEQ ID NO: 2 with an amino acid other than asparagine, and

c) a mutation that replaces the glutamine residue at amino acid number 92 in SEQ ID NO: 2 with a stop codon,

wherein the large subunit and the mutated small subunit together constitute acetohydroxy acid synthase isozyme III that catalyzes the generation of (i)  $\alpha$ -acetolactate from pyruvate and (ii)  $\alpha$ -aceto- $\alpha$ -hydroxybutyrate from  $\alpha$ -ketobutyrate and pyruvate;

and wherein L-valine feedback inhibition of acetohydroxy acid synthase activity by L-~~valine~~ is reduced to 50% or less by said mutation as compared to the unmutated sequence of the small subunit of acetohydroxy acid synthase isozyme III is SEQ ID NO: 2.

5. (Previously Presented) The isolated DNA according to claim 4, wherein the mutation at amino acid number 17 replaces serine with a phenylalanine residue, the mutation at amino acid number 29 replaces asparagine with a lysine residue or a tyrosine residue, and the mutation at amino acid number 14 replaces glycine with an aspartic acid residue.

6. (Currently Amended) ~~A~~ An isolated bacterium which harbors the DNA according to claim 1 on chromosomal DNA or plasmid in said bacterium and has an ability to produce L-valine.

7. (Currently Amended) The bacterium according to claim 6, wherein expression of said DNA is enhanced by locating said DNA under the control of a potent promoter or amplifying the copy number of said DNA.

8. (Canceled)

Application Serial No.: 09/761,782  
Supplemental Response to Office Action of May 29, 2003

9. (Original) A method for producing L-valine comprising the steps of cultivating the bacterium according to claim 6 in a culture medium, producing and accumulating L-valine in the culture medium, and collecting L- valine from the culture medium.

10. (Previously Presented) The isolated DNA according to claim 1, wherein the mutation at amino acid number 14 replaces glycine with an aspartic acid residue.

11. (Previously Presented) The isolated DNA according to claim 1, wherein the mutation at amino acid number 17 replaces serine with a phenylalanine residue.

12. (Previously Presented) The isolated DNA according to claim 4, wherein the mutation at amino acid number 14 replaces glycine with an aspartic acid residue.

13. (Previously Presented) The isolated DNA according to claim 4, wherein the mutation at amino acid number 17 replaces serine with a phenylalanine residue.

14. (Previously Presented) The isolated DNA according to claim 4, wherein the mutation at amino acid number 29 replaces asparagine with a tyrosine residue.

15. (Previously Presented) The isolated DNA according to claim 4, wherein the mutation at amino acid number 29 replaces asparagine with a lysine residue.

16. (Currently Amended) ~~A~~ An isolated bacterium which harbors the DNA according to claim 4 on chromosomal DNA or plasmid in said bacterium and has an ability to produce L-valine.

17. (Currently Amended) The bacterium according to claim 16, wherein expression of said DNA is enhanced by locating said DNA under the control of a potent promoter or amplifying a copy number of said DNA.

18. (Canceled)

Application Serial No.: 09/761,782  
Supplemental Response to Office Action of May 29, 2003

19. (Previously Presented) A method for producing L-valine comprising the steps of cultivating the bacterium according to claim 16 in a culture medium, producing and accumulating L-valine in the culture medium, and collecting L- valine from the culture medium.